IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Douglas Spencer Millar
Appl. No.	:	10/561,029
Filed	:	March 16, 2007
For	:	METHODS FOR GENOME AMPLIFICATION
Examiner	:	Thomas, David C.
Group Art Unit	:	1637

DECLARATION UNDER 37 C.F.R. §1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, Dr. Douglas Spencer Millar, declare and state:

1. I am Chief Research Scientist at Human Genetic Signatures, Pty Ltd., the assignee of the above-referenced patent application and I am an inventor on the above-referenced patent application.

2. I am an expert in the field of DNA methylation and the treatment of nucleic acids by chemical modification with sodium bisulphite. My Curriculum Vitae is enclosed (Exhibit A).

3. I am an inventor of over 20 patents, have authored 20 peer reviewed scientific papers, have contributed to 2 book chapters and have presented at more than 15 scientific meetings.

4. I am familiar with the above-referenced application, pending claims and current Office Action. I understand that the claims were rejected as allegedly being obvious over Dean et al. (*Proc. Natl. Acad. Sci. USA* 99:5261-5266, 2002) in view of Berlin (US 7,008,770), and

further in view of Olek (*Nucl. Acids Res.* 24:5064-5066, 1996), with additional references cited for some of the dependent claims (Raizis et al. *Anal. Biochem.* 226:161-166, 1995; Christensen et al., US 2006/0014144; Hogrefe et al. (US 2002/0143577). I have reviewed the pending claims and these references, and herein describe experimental data supporting the unexpected results obtained using the claimed method.

5. This Declaration is being submitted to demonstrate that the claimed invention provides unexpected advantages over the methods disclosed in Dean et al, (*Proc. Natl. Acad. Sci. USA* 99:5261-5266, 2002) in view of Berlin (US 7,008,770), and further in view of Olek (*Nucl. Acids Res.* 24:5064-5066, 1996), with additional references cited for some of the dependent claims (Raizis et al. *Anal. Biochem.* 226:161-166, 1995; Christensen et al., US 2006/0014144; Hogrefe et al. (US 2002/0143577)

6. The presently claimed method relates to treatment of genomic DNA with an agent which modifies cytosine bases, but does not modify 5'-methyl-cytosine bases, prior to amplification. This treatment effectively modifies the whole genome of the organism being investigated so that all unmethylated cytosines are converted, via uracil, to thymine and thus the modified genome comprises predominantly only 3 bases (A, G and T) instead of the native 4 bases (A, C, G and T). Modifying the genome in such a manner can be particularly advantageous when examining the genome for methylation analysis, in which the only cytosines present in the modified genome are methylated cytosines.

7. The presently claimed method allows amplification of genomic DNA which has been treated with bisulphite, acetate or citrate without substantial DNA fragmentation. The presently claimed methods facilitate the generation of long amplification products, such as amplification products which are >20 kb in size, from the treated genomic DNA. In addition, the presently claimed method allows amplification of such treated DNA from as little as 10 cells that is particularly useful in areas such as embryonic research where very limited numbers of starting material is available for analysis. These features provide significant advantages over the methods disclosed in the cited references. In particular, because the presently claimed method permits the generation of long amplification products allowing the analysis of the amplified DNA in an

unbiased manner whereas the generation of smaller fragments can lead to biased amplification of regions generated from the same genomic loci of interest but not present on the same long DNA amplicon thus resulting in an incorrect estimate of regional methylation patterns, analyses or other subsequent procedures can be conducted with a reduced number of amplification reactions relative methods which are incapable of generating long amplification products. Biases after whole genome amplification on DNA using both Bst and phi29 polymerase (which generate long DNA amplicons) are well known in the field thus the fact that the present invention can use non-fragmented DNA leads to significant advantages over methods using fragmented DNA which would exaggerate amplification bias further. Further, biased amplification using bisulphite treated DNA has previously been shown to cause incorrect calling of methylation profiles (Warnecke PM, Strizaker C, Melki JR, Millar DS, Paul CL and Clark SJ (1997). Overcoming PCR bias in quantitative methylation analysis of bisulphite-treated DNA. *Nuc. Acids Res.* **25**: 4422-4426.) thus whole genome amplification from short genomic fragments is unlikely to yield accurate quantitation of methylation levels. In addition, the claimed method allows amplification using samples containing very small amounts of nucleic acids, thereby permitting amplification in contexts where other techniques requiring higher amounts of nucleic acids in the samples would not yield results.

8. The presently claimed method provides advantages over the methods described in Dean et al. For example, in contrast to the present methods, which can, in some embodiments, be used to conduct methylation analyses, the method of Dean et al. cannot be used for methylation analysis, as there is no distinction between methylated and non-methylated cytosines.

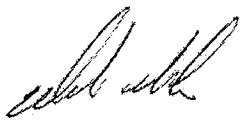
9. The presently claimed method provides advantages over the methods described in Olek et al. In the method of Olek et al., DNA is sheared or digested prior to bisulphite treatment , resulting in amplification and analysis of relatively small (<2 kb) modified genomic DNA fragments reducing whole genome coverage within the same amplicon and leading to potential biased amplification. In contrast, the present method relates to treatment of whole, undigested or unsheared genomic DNA with a bisulphite, acetate or citrate modifying agent, which converts the unmethylated cytosine bases to thymines. Thus, the present method unexpectedly allows interrogation of whole chemically converted genomic DNA, and analysis of amplification products >20 kb in size. This provides a clear and useful advantage over and above the method of Olek et

al. by interrogating long genomic regions simultaneously to determine the methylation profiles of adjacent genes on the same amplicon.

10. The presently claimed method provides advantages over the methods described in Berlin. Berlin provides a method for performing complex PCR amplification following treatment of the genomic DNA with sodium bisulphite. The fact that in Berlin a minimum of 50 type 1 primers directed towards one strand of DNA is required to successfully amplify a large portion of the genome is further evidence that the methods used by Berlin result in fragmentation of the DNA and thus amplification of relatively small modified genomic DNA fragments again leading the inherent pitfalls of biases being generated in subsequent analysis.

11. The presently claimed method provides advantages over the methods described in Raizis. Raizis teaches a method for bisulphite treatment of DNA that minimizes template degradation, including reduction of the time required to achieve completion of the bisulphite reaction using higher concentrations of bisulphite, compared to traditional methods. Raizis demonstrates amplification of a minimum of 1 pg of plasmid DNA of 6930 bp in size (5790 bp for the PGL2-Promoter plasmid plus 1140 bp neomycin gene), which is equivalent to $1.34x10^5$ copies of plasmid per PCR.. The inability of Raizis to amplify from less than 100,000 copies of plasmid indicates that their method results in a significant amount of DNA degradation. In contrast, the present invention unexpectedly allows PCR amplification of various genes from as little as 10 cells, which is a vast improvement over the method of Raizis, and indicates that the present method virtually eliminates template degradation which leads to the generation of large DNA fragments which can be analysed in a single amplicon increasing genome coverage and leading to a precise method for the analysis of methylation profiles on a genome wide scale which could not be achieved using conventional methodologies due to the limitations of amplification of short fragments that the present invention has overcome.

12. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information or belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful statements may jeopardize the validity of the application or any patent issued thereon.



11th August 2009

By: _____ Date: _____

Dr. Douglas Spencer Millar

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EXHIBIT A

CURRICULUM VITAE

Name: Douglas Spencer Millar

Tertiary Qualifications

1990-1995: Ph.D. Molecular Microbiology, St George's Hospital Medical School, Department of Surgery, University of London. Ph.D. project: *Mycobacterium paratuberculosis*, mycobacteria and chronic enteritis in humans and animals.

1986-1987: Postgraduate training, Strathclyde University, Immunology Division, Department of Bioscience and Biotechnology, 31 Taylor Street, Glasgow.

1982-1986: B.Sc. (Hons. 2.1) in Applied Microbiology, Caledonian University, Cowcaddens Road, Glasgow, G4 OBA, Scotland. Honours Project: Bacterial populations associated with *Lemna minor*.

Research and Work Experience

2001-Present Human Genetic Signatures Riverside Corporate Park, Level 4, 11 Julius Avenue, Sydney, NSW 2113. Chief Research Scientist:

1995-2001: CSIRO Division of Molecular Science, 2 Richardson Place, Delhi Road, Sydney, NSW 2113. **Senior Research Officer:**

1990-1995: Ph.D., University of London. St George's Hospital Medical School, Department of Surgery, University of London.

Jan-July 1990: Kanematsu Laboratories, RPAH, Camperdown, NSW 2050. **Research Assistant.**

1987-1989: Wellcome Diagnostics, Langley Court, Beckenham, Kent, BR3 3BS
Senior Technician, Hepatitis and new technologies section.

Publications

Frommer M, Mcdonald C, **Millar DS** *et al* (1992). 5-Methylcytosine in the kininogen gene promoter revealed by a positive strand-specific reaction. *Proc.Nat.Acad.Sci.*, USA **89**: 1827-1831.

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Priority Date 26 November 2002. International Filing Date 26 November 2003
Inventors **Douglas Millar**, John Melki, Geoffrey Grigg, George Miklos

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Priority Date 2 May 2003. International Filing Date 29 April 2004
Inventors **Douglas Millar**, Cassandra Vockler, Keralie Coulston.

Methods for Genome Amplification. PCT Application No PCT/AU2004/000722
Priority Date 17 June 2003. International Filing Date 31 May 2004. Inventors **Douglas Millar**

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Nucleic Acid Detection Assay. PCT Application No PCT/AU2004/0001196
Priority Date 4 September 2003. International Filing Date 3 September 2004
Inventors **Douglas Millar**, John Melki, George Miklos.

Assay for Detecting Methylation Changes in Nucleic Acids using an Intercalating Nucleic Acid. PCT Application No PCT/AU2004/000083. Priority Date 24 January 2004 International Filing Date 23 January 2004. Inventors **Douglas Millar**, John Melki, Geoffrey Grigg, George Miklos

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Inventor **Douglas Millar.**

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Inventor **Douglas Millar.**

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Inventors **Douglas Millar**, George Miklos, John Melki.

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Inventors **Douglas Millar**, John Melki

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Inventors **Douglas Millar**, John Melki

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Inventors **Douglas Millar**, Geoffrey Grigg

Enzymes for Amplification and Copying Nucleic Acids. Filed 30th October 2007
WO2009/067743
Inventors **Douglas Millar**, Geoffrey Grigg

Elimination Of Contaminants Associated With Nucleic Acid Amplification . Publication number: WO2009079703 (A1). Publication date: 2009-07-02
Inventors: **Douglas Spencer Millar**

Bisulphite Treatment of RNA. Publication Number: WO2009070843 (A1)
Publication Date: 2009-06-11
Inventors**: Douglas Spencer Millar**; Melki John R

Detection of Hepatitis C Virus
Australian PCT Filed 23 April 2009.
Inventor(s)**: Douglas Spencer Millar**; Bouter Nicola

EXHIBIT A

Detection of methicillin-resistant Staphylococcus aureus (MRSA)
US PCT Filed 20 April 2009.
Inventor(s): **Douglas Spencer Millar**; Inman Clare, Melki John R

Oral Presentations

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References

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